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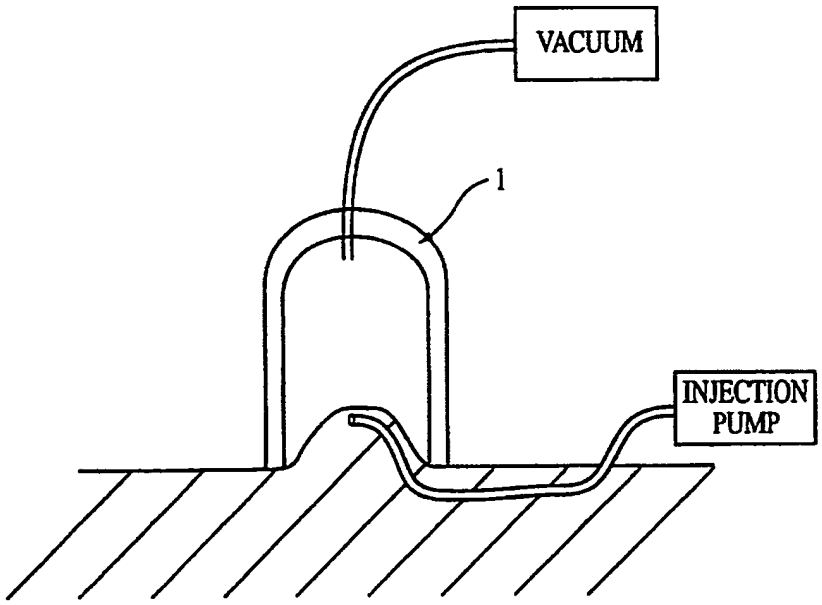

(57) Abstract

This invention is directed to methods of tissue reconstruction and kits and apparatus for the practice of the method. In the method, an injection means, which may be a hollow tube, is positioned intradermally, subdermally, or subcutaneously beneath a soft tissue defect. A tissue shaping means is positioned on top of a soft tissue defect. Conformation means is applied to conform the soft tissue defect to the shape of the tissue shaping means. Then a biocompatible material which may optionally comprise living cells is injected into a subcutaneous location to treat the soft tissue defect. A soft tissue reconstructor comprising the surface shaping means, the injection means, and the conformation means is described to facilitate the practice of the method. Further, a kit, which optionally includes a biocompatible material for injection, is described.

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SOFT TISSUE RECONSTRUCTOR AND METHOD OF USE

Background

1. Field of the Invention

The invention is directed to an apparatus for soft tissue reconstruction, methods for soft tissue reconstruction, and kits for the reconstruction of soft tissue.

2. Description of the Background

Soft tissue defects may be caused by aging, developmental disorders, surgery, trauma, and diseases such as acne and chicken pox. Such soft tissue defects may involve the face or breast and may be distressing to the patients. While superficial scarring may be treated by resurfacing procedures, the treatment of soft and distensible scarring or deep wrinkles which leave depressions on the tissue surface often require soft tissue augmentation techniques because these defects are associated with abnormalities in the deeper dermis or fat. However, surgical procedure may be inadequate for treatment where there are numerous scars proximal to each other, because performing multiple surgical procedures next to each other may cause some complications, such as tissue shrinkage and dehiscence.

In addition to correction of scarring and depressions, soft tissue augmentation is also useful for reconstructive surgery to replace protruding soft tissues lost in areas such as the breast and the face. Examples of such areas include nipples, ear lobes, noses, and lips.

One major area is a patient that may benefit from soft tissue reconstruction is the breast. More than 180,000 women are diagnosed with breast cancer per year and a majority will be treated by mastectomy or lumpectomy. Breast reconstruction following a mastectomy is a multistage procedure which can involve multiple surgeries. While some studies indicate the breast reconstruction has positive value in helping patients recover the distress of loss, many patients refuse nipple reconstruction because after multiple operations to remove the tumor and reconstruct the breast, patients may be reluctant to face additional surgery. In other cases, patients may have financial difficulties if nipple reconstruction is not covered under health insurance.

Overall, it is apparent that the known methods of soft tissue augmentation contain inherent limitations such as (1) partial or complete necrosis of the reconstructed area, (2) infection inside the reconstructed area, (3) infection of the sutures, (4) pain associated with grafting skin from other areas of the body, (5) dehiscence, (6) eventual loss of shape and elevation of the reconstructed area, and (7) high cost associated with invasive surgery. Therefore, there remains a need for improved methods for soft tissue augmentation and/or reconstruction.

Summary of the Invention

The present invention overcomes many of the limitations, problems and disadvantages associated with current strategies and designs for soft tissue reconstruction and/or augmentation and provides methods and apparatus for the treatment of soft tissue defects and soft tissue augmentation.

As embodied and broadly described herein, the present invention is directed to a method of soft tissue reconstruction and an apparatus for the practice of the method.

An object of the present invention is to provide a method of soft tissue augmentation that may be performed in an outpatient basis under local anesthesia and which avoids the complications and expense of general anesthesia.

Another object of the invention is directed to a method of soft tissue augmentation that avoids sutures and their associated complications such as scarring and increased likelihood of infection.

Another object of the invention is directed to a method of soft tissue augmentation with reduced cost.

Another object of the invention is directed to a method of soft tissue augmentation useful for treating wrinkles and folds which are due to aging or overexposure to ultraviolet radiation.

One embodiment of the invention is directed to a soft tissue reconstructor for treating soft tissue defects such as developmental defects or defects due to diseases such as chicken pox, or defects due to trauma such as accidental trauma or surgical extirpation. The patient may be any mammal, such as, for example a human.

Another embodiment of the invention is directed to a method for altering a superficial shape of a patient at a modification site. In the method, a hollow tube is inserted into the body at an insertion site so that the end of the tube is positioned subcutaneously at the desired modification site. Then a surface shaping means, such as a rigid surface is placed externally to the body at the modification site. In the method, a seal between the skin and the surface shaping means may be formed by pressing the surface shaping means against the skin. Optionally, the seal may be formed by applying a substance such as water, gels, oil or an emulsion or mixture of these substances, to the surface shaping means or the skin. A vacuum is applied through the surface shaping means until the skin and the surface shaping means are in contact (i.e., the skin conforms to the contour(s) of the surface shaping means). Finally, a biocompatible material is injected through the hollow tube into the subcutaneous location at the modification site. The hollow tube may be a needle, a cannula, a cannula with a trocar, a catheter or an angiocatheter. The conforming step may be performed before the injection step. Alternatively, the conforming step may be performed simultaneously with the injection step.

The method may be used to treat a soft tissue defect such as a skin defect. Examples of skin defects include an acne scar, a developmental defect, a skin depression, a wound scar, a surgical scar, and a surgically caused surface depression. In another embodiment of the invention, the method may be used for nipple reconstruction.

Suitable materials for use as the injectable material in the method include collagen, silicone a fragmented polymer matrix, an alginate matrix, a hydrogel matrix, ceramic beads, crushed gels and combinations and mixtures thereof. The material may optionally be biocompatible, biodegradable or both. In addition, the material for injection may comprise living cells. Preferable, the cells are autologous cells. Cell types suitable for use may be any cell that can fill a soft tissue defect from the body. Examples of suitable cells include fibroblast, myoblast, chondrocyte, endothelial cell, and vascular support cell and mixtures of these cell types.

In another embodiment of the invention, the cells contained in the injected material may be genetically altered to express a desirable characteristic. Desirable characteristics may include an altered or reduced expression of histocompatibility antigens. Another desirable characteristic is the expression of angiogenesis factors. Another desirable characteristic may be the expression of a therapeutic substance unrelated to the soft tissue defect, such as, for example, the expression of insulin for a diabetic patient.

Post injection, the patient may be treated with standard post-operative treatment such as local antiseptic treatment for the wound from the puncture of the injection means.

In another embodiment, the injection site is treated with tension releasing means after the injection. Depending on the amount of soft tissue augmentation, the skin at the injection site may be under substantial stress and tension after the injection. The stress and tension may cause the injected material to migrate away from the site of the soft tissue defect. The soft tissue defect may be treated with tension and stress releasing procedures such as shallow sutures, wound clips to assist the reconstructed site maintain its shape.

Another embodiment of the invention is directed to a soft tissue reconstructor, an apparatus for treating a soft tissue defect in a patient, which may be a mammal such as a human, comprising a surface shaping means with a surface of defined shape, a conforming means for deforming a soft tissue with the soft tissue defect to approximate the shape of at least a portion of the surface shaping means, and an injection means positioned for injecting a tissue forming composition proximal to the soft tissue defect while the soft tissue is deformed to approximate the shape of at least a portion of said surface shaping means. The conforming means is attached to the surface shaping means by an attachment means.

A portion or all of the surface shaping means may be made from a low friction material such as Teflon. In addition a portion or all of the surface shaping means may be made from a transparent or translucent material. The surface shaping means may also be a rigid surface shaped to conform to a desired exterior contour of the patient.

The desired exterior contour may be, for example, all or a portion of the contour of a nipple, a nose, an ear, a lip, or a portion of a face.

The surface shaping means may be adapted to form a vacuum seal with an exterior surface of a patient. Depending on the location to be treated, the surface shaping means may be a flat surface (see Figure 4), a curved surface, or a chamber. In the case where the surface shaping means is a chamber, the chamber may further comprise a number of inserts which can be placed within the chamber (see Figure 3). Each insert may adjust the interior volume of the chamber to conform to a desired exterior contour of a patient. For example, the insert may correspond to different sized or shaped nipples, or noses. The insert may be porous and allow the passage of air.

A porous insert is preferred when the conformation means is, for example, a vacuum source or a connection to a vacuum line which is connected to the surface shaping means. The vacuum source may further be connected to a vacuum regulator to limit the vacuum within a specific range.

In an embodiment, the injection means may be a needle, a cannula, a cannula with a trocar or a catheter. In another embodiment, the soft tissue reconstructor may have an injection means which incorporates a mechanism for mechanical positioning the injection means. One advantage of a mechanical positioning is to insure reproducible injection and placement of the tissue forming composition.

Another embodiment of the invention is directed to a kit for soft tissue reconstruction. The kit may contain, for example, a surface shaping means having a shape that conforms to at least a portion of a desired contour of an exterior surface of a body and an injection means, such as a hollow tube, for depositing a tissue forming composition into tissue. The surface shaping means may be flat, or in the shape of a chamber. If the surface shaping means is a chamber, the chamber may have an interior volume in the shape of a nipple or the interior shape and volume of the chamber may be adjusted by one or more inserts. The surface shaping means of the kit may be transparent or translucent. More typically, the surface shaping means may be made of a low friction material.

Further, the surface shaping means of the kit may be adapted for connection to a vacuum source for applying a vacuum to an exterior surface of a body. For such purposes, the kit may further comprise attachment means to connect the surface shaping means to a vacuum source.

In another embodiment of the invention, the kit comprises a surface shaping means which is coated with an adhesive to allow the attachment of the skin in the absence of vacuum.

The injection means of the kit may be any injection means as discussed above such as a needle, a cannula, or a cannula with a trocar, a catheter, or an angiocatheter. The kit may further comprise a mechanical positioning means for position said injecting means to insure reproducible placement of the tissue forming composition.

Other embodiments and advantages of the invention are set forth, in part, in the description which follows and, in part, will be obvious from this description and may be learned from the practice of the invention.

Description of the Drawings

Figure 1 depicts four reconstructed nipples at the post injection stage.

Figure 2 depicts a schematic view of a soft tissue reconstructor in use.

Figure 3 depicts a surface shaping means comprising a chamber with a plurality of inserts.

Figure 4 depicts a schematic view of the treatment of a wrinkle and an acne scar using a soft tissue reconstructor.

Figure 5 depicts one embodiment of a soft tissue reconstructor.

Figure 6 depicts one embodiment of a surface shaping means of a complex shape.

Figure 7 depicts the use of a balloon catheter like surface shaping means in the practice of the invention.

Description of the Invention

This invention is directed to methods for altering the superficial shape of a patient and apparatus for use in the method. The method may be useful for treatment involving any type of soft tissue reconstruction or augmentation. Soft tissue reconstruction may be used for the treatment of many defects such as, for example,

reconstruction of the nipple, ear, face or nose. Reconstruction may be desired to treat trauma from accidents or surgery. Other defects that may be treated include scars and depressions caused by surgery. For example, nipple reconstruction is often needed following mastectomy and nose reconstruction is often desired after surgery to remove neoplastic skin tissue from the nose. Depression of soft tissue may be also caused by removal of organs and subdermal matter for medical reasons. The method may be used to treat facial lines, creases, and wrinkles which are a regular sign of aging. These features are commonly known as frown lines, cheek depressions, vertical lip lines, marionette lines, worry lines, crow's feet, deep smile lines (nasolabial furrows) and smile lines. The method of this invention may also be used to augment normal tissue, such as, for example in plastic surgery. Examples of normal tissues augmentation include, for example, enlargement and reshaping of nipple, nose, ear, cheeks, and lips.

Other defects that may be treated by the method of the invention include a soft tissue defect by augmentation of an interior surface of a patient. Interior surfaces may be, for example, a surface in the gastrointestinal tract, or the urogenital, a nasal surface or in the oral cavity. The soft tissue augmentation of such a surface may be needed, for example, after surgical excision of cancerous tissue or for the correction of developmental defects. One example of interior soft tissue reconstruction is the reconstruction of the vocal cords after surgery.

Method for soft tissue reconstruction:

In the method, a hollow tube is inserted into the body of the patient at an insertion site proximal to an area in need (see Figure 2) of superficial modification, such that the end of the hollow tube is positioned intradermally or subcutaneously at said modification site. A surface shaping means (Figure 2, No. 1)is positioned adjacent to the exterior surface of the soft tissue defect. The area of the soft tissue defect is deformed so that the exterior surface conforms at least partially to the superficial shape of the surface shaping means. Finally a biocompatible tissue bulking material is injected subcutaneously at the modification site through the hollow tube. Each of the

steps and the individual materials and instruments used in each step is discussed in detail below.

Placement of the injection means:

The injection means may be any hollow tube such as, for example, a needle, a catheter, a hollow cannula, or a system of a cannula with a trocar or obturator. Because the tissue bulking biocompatible implant material may contain hydrogel and cells, the injection means will typically have a sufficient inner diameter to prevent undesirable damage to the structure of the gel or the killing of the cells. In a preferred embodiment, the injection means is a hollow tube having an inner diameter of about the size of a 21-gauge needle or smaller.

In one embodiment, the injection means is a hollow needle. The needle is inserted into the skin of the patient at a distance from the site of the soft tissue defect and positioned such that the tip of the needle is located intradermally or subcutaneously beneath the soft tissue defect to be treated. A suitable distance from the defect for the insertion of the needle may be, for example, between about 0.2 centimeter to about 5 centimeters, preferably between about 0.5 centimeters and about 2.0 centimeters. The injection means may be straight, curved or of a complex shape suited for the treatment of the particular soft tissue defect.

In another embodiment, a canula may be used to open a passage to a site beneath the soft tissue defect, then a hollow needle is inserted into the passage to inject the implant. In place of a hollow needle, a hollow tube may be inserted into the passage created by the cannula. The hollow tube, which may be, for example, a catheter such as an angiocatheter or a medical grade tubing, may be flexible allowing the tip to remain in place even when vacuum is applied and the skin overlying the soft tissue defect is moved.

Application of the surface shaping means:

The surface shaping means is a surface that conforms to a desired exterior shape of a patient. The surface shaping means may be a planar shape such as a flat or curved

plane. For example, if the method of the invention is used to treat a soft tissue defect such as an acne scar or a wrinkle on the face of the patient, the surface shaping means may be a rigid plate which conforms to the desired exterior facial shape of the patient (Figure 6). For example, Figure 4 depicts a flat surface shaping mean contacted to a wrinkle and an acne scar both before and after the application of vacuum.

Alternatively, if it is desired to use the method for nipple reconstruction after a mastectomy, the desired facial shape of the surface shaping means may be that of a normal nipple. In cosmetic surgery, the surface shaping means may not necessarily conform to a normal exterior shape of the patient but may conform to an enhanced exterior shape of the patient. For example, in nipple reconstruction, the desired exterior shape may have more or less volume than the normal nipple of the patient. The surface shaping means may be any exterior shape of a patient such as, for example, the exterior shape of a nipple, a nose, an ear face, abdomen, hands, and feet. Furthermore, because some implants may undergo shrinkage, the exterior shaping means may conform to a shape to account for eventual shrinkage of the reconstructed soft tissue. Shrinkage may occur as a result of a number of factors, such as partial death of the implanted cell population, resorption, and other characteristics of the implant material or implant location.

Although typically the shaping means will be positioned against skin, the skilled clinician will of course recognize that in augmentation or reconstruction of internal soft tissue structures, the shaping means may also be positioned against a mucus membrane and other membrane forming the external surface of anatomical structures including those structures that may be accessed endoscopically. Additionally, the method of the invention may be used to treat an interior lumen surface with a soft tissue defect (Figure 7, no. 74). For example, the surface shaping means may be a balloon catheter (Figure 7) used to treat a cavity in the lining of the intestine (Figure 7, no. 71), the throat (Figure 7, no. 71), or a blood vessel (Figure 7, no. 71), or stomach (Figure 7, no. 75). The skilled clinician will also know that in the treatment of a lumen, an appropriate bypass (Figure 7, no. 72) may be necessary to avoid interruption of vital fluids (e.g., blood) or air to the body. The bypass (Figure 7, no. 72) may be a part of a balloon catheter

(Figure 7, no. 76) , or the bypass (Figure 7, no. 723) may be upstream or downstream or both from the balloon catheter (Figure 7, no. 73). A bypass may be needed to maintain vital air, blood , or fluid flow.

Construction of the surface shaping means:

The surface shaping means may be constructed with any sufficiently rigid or semirigid or flexible material with sufficient strength for the practice of the method of soft tissue reconstruction. Examples of such material include, metal, ceramic, glass, graphite, wood, plastics, hard rubber and composite and mixtures thereof. A composite surface shaping means may be, for example, a shapable metal wire mesh coated with a synthetic rubber. While opaque material may be used, especially preferred material are materials that are transparent or translucent and allow visual monitoring of the underlying soft tissue during treatment. Transparent materials are clear and allow a user to see through the material to view the underlying tissue. Translucent material may be cloudy or only semi-transparent, but should allow visual judgement of the performance of the surface shaping means. Even extremely opaque material may be used for the surface shaping means of the invention but preferably there is sufficient transparency to allow a user to visually detect contact between the surface of the patient and the surface of the surface shaping means. Examples of transparent and translucent material include glass and plastics. Many plastics are suitable for the apparatus and the methods of the invention, such as, for example, phenolics, fluorocarbons, alkyds, epoxies, silicones, aminos, vinyls, polyimides, acetals, polyesters, cellulosics, carbonates, acrylics, nylons, stryenes, ABS, olefins and rubber may be made transparent or translucent. Materials which are not sufficiently rigid may be made thicker or chemically converted to provide sufficient rigidity. For example, rubber may be made with sufficient thickness to impart mechanical strength. Alternatively rubber may be vulcanized to form a surface shaping means. Typically, the surface shaping means is made of a material which can withstand standard sterilization procedures and maintain its shape, structural strength and transparency or translucency.

Another preferred material is a low friction material such as Teflon. A low friction material may allow the conformation of the soft tissue defect to the surface shaping means with less mechanical trauma. Low friction may be achieved by other means, such as the application of a friction reducing fluid to the surface shaping means. Friction reducing fluids are any fluid that can reduce the friction between the exterior shaping means and the patient. Examples of friction reducing fluids include oils, lubricants, surfactants, detergents and the like.

The exterior shaping means may also be made by a composite of different materials. For example, the exterior shaping means may have one or more metal layers to provide rigidity and a soft porous foam layer to provide comfort to a patient during treatment. Alternatively, the exterior shaping means may be a chamber with an insert, discussed below. In addition, the exterior shaping means may be made predominantly of one opaque material.

In one embodiment, the exterior shaping means may be a material which has the properties of a thermoplastic. A thermoplastic softens when heated and hardens when cooled no matter how often the process is repeated. While it is desirable that the exterior shaping means may retain thermoplastic properties after multiple heating/cooling cycles, it is not required. It is sufficient that the exterior shaping means may be softened with heat at least one time. It is understood that the external shaping means need not be thermoplastic, but only to behave sufficiently like a thermoplastic. Thus, for example, a wax or layers of fabric impregnated with wax (which are not thermoplastics) may be useful as a thermoplastic-like surface shaping means. In the method, the user, such as a doctor, may heat and mold the thermoplastic or thermoplastic like material to conform to a desired external shape of the patient before its use. This eliminates the requirement to stock numerous external shaping means to accommodate different patients. An additional advantage of the use of materials which show thermoplastic properties is that it may be used on patients that, because of surgical or accidental trauma, have an exterior surface that is hard to fit with a standard external shaping means.

Another embodiment of the method is to use a body to cast a mold of a desired soft tissue and using that mold to reconstruct the soft tissue. For example, in the case where nipple removal is necessary (such as in a mastectomy) a surface shaping means may be molded to the nipple before surgery. After surgery and nipple removal, the surface shaping means may be used to reconstruct the nipple. The same technique may be used, for example, on the nose in cases of surgery to remove skin cancer. In another example, a surface shaping means which conforms to a desired nose shape from one person or an inanimate model may be used to reconstruct a nose of similar shape in another person.

Another embodiment of the invention is directed to an exterior shaping means which is a chamber. The chamber may be any shape such as a round cylinder, a box, an irregular shape. In one embodiment, the chamber may have a hollow interior shape of a nipple. In another embodiment, the chamber may have a standard shape adapted to receive a plurality of inserts (Figure 3). Each insert may adjust the interior space of the chamber to conform to a desired external shape. For example, the chamber may be a round cylindrical shape. Inserts, conforming to different desired external shapes of a patient, may be fitted into the round cylindrical chamber to adapt the chamber for use for different body surfaces or different patients.

Inserts may be made with the same materials as the chamber. Alternatively, the insert may be made of a soft material such as foam or silicone sheeting gel. The material may be porous to allow the application of vacuum conforming means discussed below. Alternatively, the insert may be made of a non-porous material. Channels may be made through the non-porous material through which a vacuum may be applied. In one preferred embodiment, the insert may be made of a relatively inexpensive material that may be quickly sterilized and/or disposable. A chamber with inexpensive insert material may be adapted to treat multiple patients in a cost effective and prompt manner.

The surface shaping means may be positioned above the soft tissue defect by hand or by mechanical positioners. Further, known methods of positioning devices adjacent to the skin, such as, for example, bandages, velcro, adhesive tapes, adhesives,

elastic bands, slings, belts, fasteners and the like may be used to position or maintain the position of the surface shaping means (Figure 5). More typically, the shaping means may be maintained in position by application of a continuous vacuum until the final conformation can be maintained under normal skin tension conditions.

Conforming means:

The conforming means functions to deform the soft tissue adjacent to the surface shaping means, so that the exterior surface, e.g., the skin, tends to conform to the shape of the surface shaping means. The conforming means may be any means which can contact the skin of the soft tissue defect to the surface shaping means. If the surface shaping means is coated with an adhesive (discussed below), the conforming means may be removed before injection. For example, if the soft tissue defect is an acne scar on the cheek, the conforming means may be a blunt object inserted into the mouth of the patient to push the skin into contact with the surface shaping means. Similarity, if the soft tissue to be corrected or enhanced is a nose, a blunt probe may be inserted into the interior of the nose to push the skin of the soft tissue into contact with the surface shaping means.

Preferably, soft tissue deformation produced by the surface shaping means and the conforming means will serve to direct and localize the implanted material delivered by injection. When the soft tissue in the vicinity of the soft tissue defect is deformed by application of the conforming means, the biocompatible material injected according to the method of this invention will not merely follow the tissue planes. Rather, the implant assumes the desired shape for correction of the defect, under direction of the surface shaping means.

In a preferred embodiment, the conforming means may be a vacuum source or a means to connect the surface shaping means to a vacuum source. Vacuum sources may be a hand pump or a vacuum line or a portable pump. Means connecting the surface shaping means to a vacuum source may be any known methods of connecting vacuum lines such as, for example, hoses, Luer fittings, valves, regulators, traps for fluids and air filters. It is known that application of a high vacuum to skin may cause

damage. Thus, in a preferred embodiment, the conforming means may comprise a vacuum regulator to keep the amount of vacuum within limits set by the user. The vacuum regulator may be limited mechanically or electronically to vacuum in a specific range or for a specific duration determined to be safe for the particular location of the soft tissue defect.

The method of the invention may also comprise additional sealing agents and solutions that will help the surface shaping means and the conforming means maintain vacuum seals may be used. Examples of such agents include petroleum jelly, or non-petroleum jelly (such as KY jelly), oil, grease, silicone, water, glycerol and other liquids. In use, the sealing agent may be applied to either the area of the soft tissue defect or to the surface shaping means or both. The sealing agent may be able to prevent air leaks and form a more permanent vacuum seal.

In an embodiment of the invention, the additional sealing agent may be a colored liquid such as, for example, a jelly colored dark blue. The colored sealing agent is applied liberally to the soft tissue defect to fill up all the space between the surface shaping means and the soft tissue defect. If the surface shaping means is transparent or translucent, the amount of conformation may be monitored by visually monitoring the removal of the colored sealing agent beneath the surface shaping means.

In another embodiment, the surface sealing means may be a liquid adhesive. During the application of vacuum, the liquid adhesive may prevent air leaks. Further, if the adhesive is of sufficient strength, the skin overlying the soft tissue defect may adhere to the surface shaping means after the conforming means -- the vacuum -- is removed. It is desirable that the adhesive serve as a friction reducing agent before setting and an adhesive after setting.

In a preferred embodiment of the invention, the surface shaping means is a chamber fitted with an insert which is coated with adhesives. The chamber is applied over a soft tissue defect and vacuum is applied. The skin underneath the chamber is contacted to the chamber because of the vacuum. The adhesive is allowed to set and the chamber may be removed leaving the insert attached to the patient's skin. The insert may serve to maintain the shape of the soft tissue defect for a period of time after the

procedure. Further, the insert may prevent damage to the newly restructured soft tissue by preventing trauma to the site.

So long as the combination of the surface shaping means and the conforming means act to direct the implant to the desired location and shape, it is not necessary that the surface shaping means exactly mirror the superficial shape desired for correction of the defect. For example, a simple open ended cylinder may serve to shape a reconstructed nipple with a diameter controlled by the diameter of the cylinder and height controlled by a combination of vacuum and volume of injected material.

Injection of material:

The implant composition may be drawn into the desired implant site by the vacuum created when the conforming means applies vacuum over the tissue overlying the tip of the injection means. The success of such an injection method may depend on the viscosity of the implanted material, the resistance of the tube to fluid flow, and the amount of vacuum applied at the soft tissue defect. The resistance of the tube to fluid flow may be a function of the length, diameter and curvature of the tube and the viscosity of the implanted material.

The material to be implanted may be injected into the implant site by external pressure. The injection means may comprise a positive pressure injection device, such as a syringe. A positive pressure injection device may be any device that can apply a positive pressure to fluids entering the injection tube catheter or needle. In one embodiment, the positive pressure device may be a pump which may be a reversible pump. Examples of reversible pumps include syringes, fluid displacement pumps, electrical pumps and the like. In one embodiment, a reversible pump is used both to deliver implant material to the soft tissue defect and to remove materials from the soft tissue defect. The removal of material from the soft tissue defect may be desirable if the defect is fluid filled. A reversible pump may also be useful to adjust the amount of material delivered to the site. In a preferred embodiment, the soft tissue reconstructor is used to deliver implantation material to a soft tissue defect and the reconstructor is removed except for the injection means. The corrected defect is then inspected visually

and if too much material was injected, the excess material may be removed by the reversible pump.

Material for Injection:

Material for implantation may be any natural or synthetic material used for correction, augmentation, or reconstruction of soft tissue defects such as, for example, collagen (such as that commercially available and sold under the trade names of Zyderm I, Zyderm II and Zyplast), adipose tissue, silicone, plastics, gelatin, teflon paste, and mixtures and the like. However, these materials are not permanent. Since collagen and silicone are reabsorbed with time, and autologous fat may have a long term survival rate of 10% or less, multiple treatment sessions may be necessary. Preferable materials for implantation include those materials that maintain their form after deposit. Materials that fit this category include those that comprise living tissue or material that will attract growth and colonization of living tissue into a site. Suitable materials include tissue engineering compositions which are implantable by injection, such as those described in U.S. Patent No. 5,667,778 and International Application Nos. PCT/US96/09065 and PCT/US97/22859, each of which is incorporated herein by reference.

Typically the injected material will be more or less biocompatible. However, the material to be injected need not be perfectly biocompatible. Since much of the tissue bulking effect is due to a fibrotic response, a strictly benign material may be less preferred in some cases.

One suitable polymeric material is a hydrogel. A hydrogel is defined as a substance formed when an organic polymer (natural or synthetic) is cross-linked via covalent, ionic, or hydrogen bonds to create a three-dimensional open-lattice structure which entraps water molecules to form a gel. Examples of materials which can be used to form a hydrogel include polysaccharides such as alginate, polyphosphazines, and polyacrylates, which are crosslinked ionically, or block copolymers such as Pluronics™ or Tetronics™, polyethylene oxide-polypropylene glycol block copolymers which are crosslinked by temperature or pH, respectively. Other materials include proteins such as fibrin, polymers such as polyvinylpyrrolidone, hyaluronic acid and collagen.

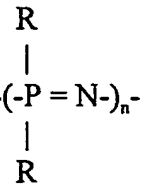
In general, these polymers are at least partially soluble in aqueous solutions, such as water, buffered salt solutions, or aqueous alcohol solutions, that have charged side groups, or a monovalent ionic salt thereof. Examples of polymers with acidic side groups that can be reacted with cations are poly(phosphazenes), poly(acrylic acids), poly(methacrylic acids), copolymers of acrylic acid and methacrylic acid, polyvinyl acetate, and sulfonated polymers, such as sulfonated polystyrene. Copolymers having acidic side groups formed by reaction of acrylic or methacrylic acid and vinyl ether monomers or polymers can also be used. Examples of acidic groups are carboxylic acid groups, sulfonic acid groups, halogenated (preferably fluorinated) alcohol groups, phenolic OH groups, and acidic OH groups.

Examples of polymers with basic side groups that can be reacted with anions are polyvinyl amines, polyvinyl pyridine, polyvinyl imidazole, and some imino substituted polyphosphazenes. The ammonium or quaternary salt of the polymers can also be formed from the backbone nitrogens or pendant imino groups. Examples of basic side groups are amino and imino groups.

Alginate can be ionically cross-linked with divalent cations, in water, at room temperature, to form a hydrogel matrix. Due to these mild conditions, alginate has been the most commonly used polymer for hybridoma cell encapsulation, as described, for example, in U.S. Patent No. 4,352,883 to Lim. In the Lim process, an aqueous solution containing the biological materials to be encapsulated is suspended in a solution of a water soluble polymer, the suspension is formed into droplets which are configured into discrete microcapsules by contact with multivalent cations, then the surface of the microcapsules is crosslinked with polyamino acids to form a semipermeable membrane around the encapsulated materials.

Polyphosphazenes are polymers with backbones consisting of nitrogen and phosphorous separated by alternating single and double bonds. Each phosphorous atom

is covalently bonded to two side chains ("R"). The repeat unit in polyphosphazenes has the general structure (1):



where n is an integer.

The polyphosphazenes suitable for cross-linking have a majority of side chain groups which are acidic and capable of forming salt bridges with di- or trivalent cations. Examples of preferred acidic side groups are carboxylic acid groups and sulfonic acid groups. Hydrolytically stable polyphosphazenes are formed of monomers having carboxylic acid side groups that are crosslinked by divalent or trivalent cations such as Ca^{2+} or Al^{3+}. Polymers can be synthesized that degrade by hydrolysis by incorporating monomers having imidazole, amino acid ester, or glycerol side groups. For example, a polyanionic poly [bis(carboxylatophenoxy)] phosphazene (PCPP) can be synthesized, which is cross-linked with dissolved multivalent cations in aqueous media at room temperature or below to form hydrogel matrices.

Bioerodible polyphosphazines have at least two differing types of side chains, acidic side groups capable of forming salt bridges with multivalent cations, and side groups that hydrolyze under *in vivo* conditions, e.g., imidazole groups, amino acid esters, glycerol and glucosyl. The term bioerodible or biodegradable, as used herein, means a polymer that dissolves or degrades within a period that is acceptable in the desired application (usually *in vivo* therapy), less than about five years and most preferably less than about one year, once exposed to a physiological solution of pH 6-8 having a temperature of between about 25°C and 38°C. Hydrolysis of the side chain results in erosion of the polymer. Examples of hydrolyzing side chains are unsubstituted and substituted imidizoles and amino acid esters in which the group is bonded to the phosphorous atom through an amino linkage (polyphosphazene polymers in which both R groups are attached in this manner are known as polyaminophosphazenes). For polyimidazolephosphazenes, some of the "R" groups on

the polyphosphazene backbone are imidazole rings, attached to phosphorous in the backbone through a ring nitrogen atom. Other "R" groups can be organic residues that do not participate in hydrolysis, such as methyl phenoxy groups or other groups shown in the scientific paper of Allcock, et al., Macromolecule 10:824-830 (1977).

Methods for synthesis and the analysis of various types of polyphosphazenes are described by Allcock, H.R.; et al., Inorg. Chem. 11, 2584 (1972) ; Allcock, et al., Macromolecules 16, 715 (1983); Allcock, et al., Macromolecules 19, 1508 (1986); Allcock, et al., Biomaterials, 19, 500 (1988); Allcock, et al., Macromolecules 21, 1980 (1988) ; Allcock, et al. , Inorg. Chem. 21(2), 515-521 (1982); Allcock, et al., Macromolecules 22, 75 (1989); U.S. Patent Nos. 4,440,921, 4,495,174 and 4,880,622 to Allcock, et al.; U.S. Patent No. 4,946,938 to Magill, et al.; and Grolleman, et al., J. Controlled Release 3, 143 (1986), the teachings of which are specifically incorporated herein by reference.

Methods for the synthesis of the other polymers described above are known to those skilled in the art. See, for example Concise Encyclopedia of Polymer Science and Polymeric Amines and Ammonium Salts, E. Goethals, editor (Pergamen Press, Elmsford, NY 1980). Many polymers, such as poly (acrylic acid) , are commercially available.

The water soluble polymer with charged side groups is crosslinked by reacting the polymer with an aqueous solution containing multivalent ions of the opposite charge, either multivalent cations if the polymer has acidic side groups or multivalent anions if the polymer has basic side groups. The preferred cations for cross-linking of the polymers with acidic side groups to form a hydrogel are divalent and trivalent cations such as copper, calcium, aluminum, magnesium, strontium, barium, and tin, although di-, tri- or tetra-functional organic cations such as alkylammonium salts, e.g., R_3N+ -VVV-$^{+}NR_3$ can also be used. Aqueous solutions of the salts of these cations are added to the polymers to form soft, highly swollen hydrogels and membranes. The higher the concentration of cation, or the higher the valence, the greater the degree of cross-linking of the polymer. Concentrations from as low as 0.005 M has been

demonstrated to cross-link the polymer. Higher concentrations are limited by the solubility of the salt.

The preferred anions for cross-linking of the polymers to form a hydrogel are divalent and trivalent anions such as low molecular weight dicarboxylic acids, for example, terepthalic acid, sulfate ions and carbonate ions. Aqueous solutions of the salts of these anions are added to the polymers to form soft, highly swollen hydrogels and membranes, as described with respect to cations.

A variety of polycations can be used to complex and thereby stabilize the polymer hydrogel into a semi-permeable surface membrane. Examples of materials that can be used include polymers having basic reactive groups such as amine or imine groups, having a preferred molecular weight between 3,000 and 100,000, such as polyethylenimine and polylysine. These are commercially available. one polycation is poly(L-lysine); examples of synthetic polyamines are: polyethyleneimine, poly(vinylamine), and poly(allyl amine). There are also natural polycations such as the polysaccharide, chitosan.

Polyanions that can be used to form a semi-permeable membrane by reaction with basic surface groups on the polymer hydrogel include polymers and copolymers of acrylic acid, methacrylic acid, and other derivatives of acrylic acid, polymers with pendant SO_3H groups such as sulfonated polystyrene, and polystyrene with carboxylic acid groups.

Biocompatible Material comprising cells:

The biocompatible injection material may optionally comprise cells. Cells may be collected by biopsy or purchased commercially from suppliers such as American Tissue Type Collection (Manassas VA). Autologous or allogeneic cells may be collected from many sites, including chondrocytes from auricular cartilage and fibroblasts from skin. Collection may be performed using standard techniques such as, for example, by the use of a biopsy gun. After collection, cells may be processed and placed into, e.g., a cell/alginate suspension. The cell suspension may be implanted using the soft tissue reconstructor described below.

Examples of cells which can be implanted as described herein include chondrocytes and other cells that form cartilage, osteoblasts and other cells that form bone, muscle cells, fibroblasts, and organ cells, and also include expanded populations of isolated stem cells. As used herein, "organ cells" includes hepatocyte, islet cells, cells of intestinal origin, cells derived from the kidney, adipocytes, endothelial cells and other cells acting primarily to synthesize and secret, or to metabolize materials.

The cells to be encased in the injectable hydrogel can be obtained directly from a donor, from cell culture of cells from a donor, or from established cell culture lines. In the preferred embodiments, cells are obtained directly from a donor, washed and implanted directly in combination with the polymeric material. Cellular material may be dissociated single cells, minced tissue (i.e., clumps of aggregated cells) or cell aggregates generated *in vitro* from dissociated cells. The cells may be cultured using techniques known to those skilled in the art of tissue culture.

In a preferred embodiment, cells of the same species and preferably the same or similar immunological profiles are obtained by biopsy, either from the patient or a histocompatible donor such as a close relative. The cells are then grown to confluence in culture using standard conditions as described in the Examples section. If the cells are commercially purchased, such as, for example, from American Tissue Culture Collection (Manassas VA), then the cells may be grown according to the instructions form the supplier. If cells that are likely to elicit an immune reaction are used, such as human muscle cells from immunologically distinct individual, then the recipient can be immunosuppressed as needed, for example, using a schedule of steroids and other immunosuppressant drugs such as cyclosporine. However, in the most preferred embodiment, the cells are autologous.

It is understood that precursor cells, that is, cells that have not undergone terminal differentiation may be also used. Examples of precursor cells include fibroblasts which may differentiate to form chondrocytes. As described herein, the term "cells" includes mature cells and precursor cells which have not undergone terminal differentiation.

In a preferred embodiment of the invention, cells are suspended in a transplantation matrix polymer solution before transplantation. One preferred matrix for suspending cells is a hydrogel. Substantial variation is permitted in the order of addition for the components which react to form the cell-containing hydrogel. Generally, the cells are added before the hydrogel reaches a partially hardened consistency. Typically this means that the cells are introduced into a premixture containing either the alginate or one or more of the cation sources before the cation sources and the alginate are combined. Usually cells are first mixed with alginate, optionally including sequestering anions, and then the cation sources are added in one or more steps. In one example, chondrocytes, or other suitable cells, are harvested, grown to confluence, passaged as needed, then mixed with a biodegradable liquid polymer such as alginate which is designed to solidify at a controlled rate upon subsequent mixing with multivalent ionic salts. Variations in the levels of individual components, or conditions of mixing and incubation can be modified to a) control the consistency of the material as injected; b) control the time required for attaining an injectable consistency; c) and controlling the properties of the final gel *in vivo* to accommodate particular requirements of the transplanted cells for engaftment and function or of the receiving tissue site for appropriate texture and dimensional retention.

Levels of individual components, either singly or in combination, can be modified to alter different properties of the formulation both before and after application so as to accommodate particular requirements a) for injection and application, b) for individual cell viability or successful engraftment and creation of required properties and function of the final gel and replacement tissue, or c) for the manufacture, distribution, and application to patients of the formulation. For example, injection of a cell/gel formulation into compact tissues (e.g. muscle, submucosa) requires a high viscosity to prevent extravasation of material, while application to achieve a large surface area distribution (peritoneal injection) or to coat preformed structural elements (vascular grafts, stents, polymer scaffolds, etc.) requires a solution of lower viscosity. Alterations of viscosity can be achieved by a number of mechanisms either singly or in concert, such as (1) selection of the viscosity of the raw material (e.g.,

low, medium, or high viscosity alginate); (2) concentration of gel (e.g., a range of 0.3% to 3.0% alginate can be used to achieve a broad range of gel viscosities); (3) amount of highly soluble multivalent cation source to control degree of partial cross-linking; or (4) level of anions provided to compete with alginate for cation binding. Successful engraftment of different cell types, or the nature of the desired replacement tissue to be created may require alterations of the formulations components. For example, creation of a firm, compact tissue (e.g., liver, cartilage) requires a gel of higher polymer chain length or concentration (e.g., >1.5% alginate, high guluronic content alginate), while simply coating a surface such as bone surface augmentation using a gel/osteoclast formulation is more successful with a lower concentration of polymer.

Controlling the duration of injectability and properties of a formulation prior to injection will aid the manufacture, distribution, and application of gel/cell therapies. The working time of the final formulation, or the time required for materials to setup to an injectable consistency, can be controlled through multiple methods, such as by the amount of highly soluble cation provided and/or the level of anion provided to compete for cation binding, in addition to temperature regulation. For example duration of an injectable consistency can be controlled through temperature so as to allow a working time of minutes (34-38°C) or beyond one month (4-8°C). Temperature plays a very important role in the gel formation speed. Upon increasing the temperature, as will occur, for instance, when the material is injected into tissue having a temperature of 37°C, the material will proceed to harden into a fully cross-linked hydrogel. At $CaSO_4 \cdot 2H_2O$ level of 0.1 x (i.e. 20 mg/ml in a formulated batch) the partially hardened gel could set up in 32 minute at body temperature and remains injectable for at least 90 minutes. This best case scenario of $CaSO_4$ at 37 °C still requires too much time to reach injectable consistency, and is both more complicated and more inconsistent than desired.

Cation sources can be chosen to control gelling and gel properties, and also combined to control degradation. The composition according to this invention, having both a fast and slow source of cross-linking agents, can provide both more rapid viscosity increase, and a longer time before becoming fully cross-linked compared to

the formulation with calcium sulfate alone. In a particularly preferred mode, the mixture which forms partially hardened hydrogel will also include a significant amount of phosphate anion. Typically, the phosphate concentration will be approximately 0.1 molar.

Increasing the concentration of alginate is also helpful for the gel to hold more water, resulting in a thicker paste and a stronger gel, and also in accelerating the gel formation. Preferred alginate level is at least 0.75%, more preferably between 1.5% and 2.5% and up to 3.0%, in the final cell-containing suspension which forms the hydrogel.

In a preferred embodiment of this invention, the acidic biopolymer is sodium alginate, the fully soluble salt is calcium chloride, and the sparingly soluble salt is calcium sulfate. The alginate is present in the amount of at least 0.75% by weight of solution, preferably about 1.5%. The freely soluble salt provides 10 to 15 mM calcium ion, while the sparingly soluble salt is provided as a powder dispersed in the alginate solution in an amount which would supply approximately 8 times more calcium ion if it were completely dissolved. In other words, calcium is supplied as the divalent metal cross-linking agent by two salts, calcium chloride and calcium sulfate, in a ratio of 1 part to 10 parts by weight. In a preferred method of preparing the hydrogel suspension, 9 parts of a 2% alginate solution are mixed with 2 parts of a cell suspension in a suitable cell suspension medium such as M-199, and subsequently 1 part of the same cell suspension medium containing 1.8% anhydrous calcium chloride with 18% calcium sulfate suspended therein is added to the alginate-cell mixture. The final mixture is mixed thoroughly, taking adequate precautions to avoid damaging the cells, and used for injection into tissue. A rapid increase in viscosity may be expected within 15 minutes of mixing all of the components together. The consistency of this mixture should be sufficient for the mixture to hold its shape against gravity, but will remain injectable at room temperature for at least 24 hours.

In one preferred embodiment described herein, calcium alginate or certain other polymers that can form ionic hydrogels which are malleable are used to encapsulate cells. The hydrogel is produced by cross-linking the anionic salt of alginic acid, a carbohydrate polymer isolated from seaweed, with calcium cations, whose strength

increases with either increasing concentrations of calcium ions or alginate. Then the suspension is injected directly into a patient prior to hardening of the suspension. The suspension then hardens over a short period of time due to the presence in vivo of physiological concentrations of calcium ions.

A preferred viscosity for injectable application varies by intended use, but is a balance of parameters that include (a) the formulation reservoir (e.g., syringe size, piston diameter, resistance required for proper "touch" or application rate), (b) application device (catheter or needle length, diameter, composition), (c) receiving tissue resistance to division, and (d) type of distribution required (e.g., topical application to bone or organ surface, injection within tissues without extravasation, etc.). In one embodiment, the composition is formulated to form a partially hardened hydrogel within thirty minutes, preferably within 10-15 minutes at room temperature, the consistency of the partially hardened hydrogel having a viscosity of at least 15,000 centipoise at 25°C, or alternatively being fluid enough for injection through a 23-gauge cannula at a rate of about 10 ml/minute without substantially damaging the cells (i.e., viability of the cells reduced by no more than 25%).

Typically, cells are suspended in a hydrogel solution and injected directly into a site in a patient, where the hydrogel hardens into a matrix having cells dispersed therein. Alternatively, cells may be suspended in a hydrogel solution which is poured or injected into a rigid or inflatable mold having a desired anatomical shape, then hardened to form a matrix having cells dispersed therein which can be implanted into a patient. After implantation, the hydrogel ultimately degrades, leaving only the resulting tissue. Such hydrogel-cell mixtures can be used for a variety of reconstructive procedures, including custom molding of cell implants to reconstruct three-dimensional tissue defects, filling pre-inserted inflatable molds or scaffolds, as well as implantation of tissues generally.

Another embodiment of the invention is directed to the use of a fractured hydrogel. Injection of alginate gel into subcutaneous sites promotes an infiltration of loose connective tissue in and around the injected gel fragment. Preferably the hydrogel is a friable material which fractures when forced through an orifice such as a syringe

needle or cannula such as the alginate gel described in the previous section. The fractured hydrogel will be a suspension of irregularly shaped particles whose size is a function of the orifice size and pressure. Typically, the size of a fractured hydrogel will range from 40 um to about 300 um. The suspension will retain sufficient consistency (viscosity) to divide tissue when injected and to avoid extravasation from the injection site. Suitable orifice size and injection pressures can be determined by the skilled artisan, and effective particle sizes can be confirmed by monitoring the fibrotic response.

Typically, a hydrogel solution is injected directly into a site in a patient, where the hydrogel forms an implant and induces fibrotic response from the surrounding tissues. The loose connective tissue formed by the fibrotic response holds the hydrogel particles in place, and tissue formation is accompanied by vascularization, producing vascularized mass of soft tissue consistency occupying the volume established during implantation. Such hydrogel implants can be used for a variety of reconstructive procedures, including molding of implants *in situ* to reconstruct three-dimensional tissue defects.

In another embodiment of the invention, the polymeric matrix can be combined with humoral factors to promote cell transplantation and engraftment. For example, the polymeric matrix can be combined with angiogenic factors, antibiotics, antiinflammatories, growth factors, compounds which induce differentiation, and other factors which are known to those skilled in the art of cell culture. For example, humoral factors could be mixed in a slow-release form with the cell-alginate suspension prior to formation of implant or transplantation. Alternatively, the hydrogel could be modified to bind humoral factors or signal recognition sequences prior to combination with isolated cell suspension.

While individual desirable characteristics of an injectable material is individually described above, it is understood that a suitable injectable material of the invention may comprise any one, or combination of all of the discussed material. For example, a therapeutic substance may be encapsulated in a hydrogel and mixed with cells, silicones. cholesterol, and injected in a method of the of the invention.

Injection:

As described herein, an injectable biodegradable polymer which optionally comprise a cell population is injected into a site for augmentation or reconstruction of a soft tissue defect. This method may be used, for example, for the treatment of acne scars, or facial furrows. Alternatively, this method may be used to reconstruct new soft tissue structures to replace tissues lost to surgery, such as, for example, nipples lost in a mastectomy. In another embodiment, the method may be used to treat a soft tissue deformity such as inverted nipples, muscle atrophy due to congenital or acquired diseases or secondary to trauma, burns, and the like.

The suspension can be injected through a hollow tube such as a catheter, needle, hollow cannula, or a cannula with a trocar. Because the cells are injected subcutaneously, the position of the injector may be monitored by direct palpation or visual monitoring. Visual monitoring is especially useful if the hollow tube is of a color not normally found on human skin, such as, for example, orange. The suspension could also be injected through a catheter or needle with fluoroscopic, monographic, computed tomography, magnetic resonance imaging or other type of radiologic guidance. The material might be injected after an initial pretreatment of the tissue with an agent such as collagenase to loosen the intracellular tissue to permit an easier shaping of the reconstructed tissue.

One advantage of the cell suspension is that the reconstructed tissue is permanent and does not require periodic reimplantation. Alginate-bovine chondrocyte cell allografts may contain viable cartilage cells after implantation times for as long as 90 days in athymic mice. The new cartilage formed retains the approximate configuration and dimensions of the injected template. The cell-polymer construct is essential in that injection of free chondrocytes or alginate alone does not result in cartilage formation. The time to solidification of the alginate-cell solution may be manipulated by varying the concentration of calcium as well as the temperature at which the chondrocytes are added to the alginate. The use of autologous chondrocytes precludes an immunologic reaction against the cells.

One disadvantage of traditional methods of injection is that there is little control over the site distribution of material injected intradermally or subcutaneously. Materials injected by simple injection divide the tissue plane along the path of least resistance, generally producing a generally circular deposit of material. The method of the invention, when used in concert with placing the tip of the injection device within the area defined by the vacuum, acts to preferentially localize the injected material to the area defined by the vacuum. The area defined by vacuum can be altered in size by using various application instruments. Results of such defined areas of application are either a precise lifting of a depressed area in skin, such as acne scars, to the normal skin plane, or produce an elevation of tissue of defined shape and height, such as a mammary nipple.

Post injection, the tissue shaping means, the conformation means, and the injection means may be removed and the reconstructed soft tissue can be allowed to heal normally. Normal post-surgical treatment, such as protection from compression and a course of antibiotics may be optionally followed.

While the method of soft tissue reconstruction involving the use of one tissue shaping means, one conformation means, and one injector means is described, it is understood that the method of the invention may also be practice with any number of each in combination. Thus, for example, a large soft tissue defect may be treated with 2 or more injection means but one conformation means and one tissue shaping means. Alternatively, a complex reconstruction may require 3 surface shaping means connected to one conformation means and injected with 4 injection means. The exact combination to use will depend on the patient and the type of soft tissue defect.

The method of the invention may also be used for delivery of a therapeutic substance to a patient. In this embodiment, a hollow tube is placed subcutaneously or subdermally at an injection site of a patient. A surface shaping means is placed on top of the injection site and a conformation means is applied to the injection site. A therapeutic substance is injected into the injection site. The therapeutic compound is delivered locally rather than systemically. Typically the therapeutic substance will be incorporated in a biocompatible carrier, which may optionally include a slow release

carrier. A slow release carrier may be any carrier known to delay release of a substance. Examples of such carriers include biodegradable or non-biodegradable plastics, gels, hydrogels, ceramics and silicone which encapsulate or entrap a therapeutic substance.

After injection, the therapeutic substance appears as an elevated portion of soft tissue in a patient. One advantage of the method is that it allows a visual conformation of the placement of the therapeutic substance. The therapeutic substance will be a biologically active material of desired activity which may be a naturally occurring substance or a non-natural substance produced synthetically, semi-synthetically, recombinantly, or the like. The therapeutic substance may be, for example, a contraceptive, an antipsychotic drug, or a slow release insulin for diabetes. In such cases, the method of the invention allows quick visual conformation of the application of the therapeutic substance. Another advantage of the treatment is the localized nature of the therapeutic substance. If an adverse reaction to the therapeutic substance develops or if the health care provider or patient choose to discontinue treatment, the localized therapeutic substance may be removed rapidly with minimal trauma to the patient. In a preferred embodiment, the carrier may be a biodegradable carrier which can alleviate the need for future removal. Another advantage of the treatment is the ability to deliver a concentrated dosage of a therapeutic substance locally rather than systemically. Thus, a therapeutic substance which may be delivered locally at a concentrations that may be toxic if administration was systemic. This method may be used, for example, to treat an inoperable skin sarcoma by positioning a highly localized dose of an anti-neoplastic agent.

Soft Tissue Reconstructor:

One embodiment of the invention is directed to a soft tissue reconstructor for use in practice of the methods of the invention. The soft tissue reconstructor has a surface shaping means, a conforming means, an injection means. The soft tissue reconstructor may further contain mechanical aids to assure the proper positioning of the injection means. Mechanical aids may include markings on the injection catheter

to indicate the length of insertion to guides and alignment devices to assist in the positioning of the surface shaping means and the injection means.

An example of one configuration of a soft tissue reconstructor is depicted in Figure 5. In Figure 5, a surface shaping means **1**, is connected to conformation means **2**. Conformation means **2** comprise a connector to a vacuum source **4**. A vacuum regulator 3 is attached to the conformation means **2**. A reversible pump **9** is used as an injection means **11**. The reversible pump further comprises regulator means **8**, to control the amount of material injected. A guide **7** allow a visual feedback of the amount of insertion of the hollow tube **10**, of the injection means **11**. Adhesive tape attachment means **6**, is used to secure the soft tissue reconstructor to the skin **12** of a patient by a flange **5**.

Embodiments of a soft tissue reconstructor includes the materials and structures described above. In one embodiment, a soft tissue reconstructor may comprise a surface shaping means attached to a conformation means. The soft tissue reconstructor may comprise a chamber with can be fitted with a plurality of inserts so adapt the chamber for a variety of soft tissue reconstruction. The soft tissue shaping means may further incorporate mechanical aids to hold and guide the injection means. For example, the soft tissue shaping means may comprise a detector to detect the tip of the injection means. The detector may, for example, detect a magnetic component incorporated in the tip of the injector means. In operation, the detector may guide the injection tip to the proper location. Further, if during the procedure, the injection means should become displaced, the detector may sound an alarm to notify the user to stop the procedure. The user can then reposition the tip of the injection means with the help of the detector or abort the procedure.

In an embodiment of the soft tissue reconstructor, a vacuum gauge may be incorporated into the vacuum connection or a pressure gauge may be incorporated into the tissue shaping means to detect the amount of vacuum or pressure developed at the site during the procedure. The injection of an adequate amount of material may be detected by the detector and a signal may be communicated to the user to stop the procedure. This feature is especially useful, for example in the reconstruction of both

nipples of a patient to achieve nipples of the same shape and size. This feature may also be used, for example, in the reconstruction of multiple acne scars to achieve a uniform and desirable effect.

Kit:

Another embodiment of the invention is directed to a kit to practice the method of the invention. A kit may comprise, for example one or more of a surface shaping means, a conformation means, an injection means in a package. The package may be sterile to facilitate immediate use by the end user. The kit may optionally further comprise biocompatible material for implantation into a patient.

Other embodiments and advantages of the invention are set forth, in part, in the description which follows and, in part, will be obvious from this description and may be learned from practice of the invention.

Examples:

Example 1 Isolation of Cells for Implantation:

Cells such as muscle cells and fibroblast are obtained from each animal or from a commercially source such as the American Tissue Type Collection (Manassas VA). Cells were harvested and plated separately in vitro using standard cell culture techniques. Briefly, Cartilage obtained from animals were washed in providine-iodine 10 percent solution and chondrocytes were harvested under sterile conditions using a technique described by Klagsbrun, "Large scale preparation of chondrocytes" Methods in Enzymology, 58:560 (1979). The isolated cells were quantitated using a hemocytometer, and the chondrocyte suspension was concentrated to 20, 30, and 40 x 10^6 cells per ml.

Example 2 Formation of Cell-Calcium Alginate Suspension.

Two percent weight/volume sodium alginate (0.1 M K_2PO_4, 0.135 M NaCl, pH 7.4, Protan, Portsmouth, NH) was made and sterilized in ethylene oxide. A 1.5 ml aliquot of $20X10^6$ cells/ml bladder muscle cell suspension was added to an equal volume of sodium alginate solution for a final alginate concentration of 1%. The

bladder muscle cell-sodium alginate suspension is kept at 32°C. Immediately prior to injection, calcium sulfate (0.2 g/ml) was added to the bladder muscle cell-sodium alginate suspension. The mixture is vortexed and stored in ice until injection. The gelling process was initiated with the addition of calcium sulfate, which allowed the suspension to remain in a liquid state for approximately 40 minutes.

Example 3 Implantation.

Mini-pigs were anesthetized with intramuscular injections of 25 ml/kg ketamine and 1 ml/kg acylpromazine. Additional anesthesia was obtained with an intramuscular administration of 25 mg/kg ketamine and 10 mg/kg of xylazine. Animals were placed in a supine position. A catheter attached to a syringe filled with an alginate or a cell/alginate suspension is introduced subcutaneously until the tip of the catheter is about 4 cm subcutaneous from the site of entry. About 1 ml of the alginate solution is injected . Approximately 2 ml of the autologous cartilage-alginate suspension (40-60 x 106 chondrocytes) were injected through the needle, while vacuum was applied continuously.

Example 4 Formation of Nipples in Pigs.

Eight or nine nipples were created on the abdomens of each of five adult market pigs. In one pig, all eight nipples were formed from calcium alginate implantation alone. In two pigs, nipples were created from calcium alginate alone (n=9), or from calcium alginate containing cultured autogenous chondrocytes obtained from the animal's auricular cartilage during a prior surgical procedure -- either 20 million cells per ml (n=9) or 30 million cells per ml (n=9). In these initial three animals, a purse string technique was used.

In a fourth pig, nipples were created using a purse string method (n=5) or a sutureless vacuum technique (n=5) using alginate plus 30 million cells per ml. In a final pig, nipples were all created using the vacuum technique employing either Pluronic plus 30 million cells per ml (n=4) or calcium alginate plus 30 million cells per ml (n=4). Measurements of each nipple were taken at the time of injection and at weekly intervals thereafter until ten weeks with photographic and video documentation of each nipple at each session. Histological evaluation of all specimens was carried out following the

final measurement session. Three fresh human abdominal tissue specimens were used to assess whether nipples of similar form to those created in the pigs could be generated and to permit histologic comparison of dermal thickness in pig and human abdominal skin.

As seen in Figure 1, at twelve weeks, there was a statistically significant decrease in both the normalized projection and the normalized volume of nipples created from calcium alginate alone and of nipples created from calcium alginate containing 20 million chondrocytes per ml. In contrast, nipples created from calcium alginate containing 30 million chondrocytes per ml maintained a ten millimeter normalized projection over ten weeks. The sutureless vacuum method was easier to use and provided more consistent results. Dermal thickness of human and pig abdominal skin was found to be comparable by light microscopy.

Example 5 Isolation and Culture of Chondrocytes

Chondrocytes obtained by biopsy, for example, from joints or rib regions, are harvested and cultured, passaging as necessary to remove contaminating non-chondrocytes. The cartilage is sterilized by washing in Povidone-Iodine 10% solution (Betadine, Purdue Frederick Co., Norwalk, Conn.). Then under sterile conditions, the muscle attachments are dissected from the underlying bone to expose the joint surfaces. The cartilage from the articulating surfaces of the joint is then sharply dissected from the underlying bone. The cartilage is cut into pieces with dimensions of less than 5 mm per side and washed twice in Phosphate Buffered Saline (PBS) with electrolytes and adjusted to neutral pH. The minced cartilage is then incubated at 37°C in a solution of 0.2% clostridial collagenase (Worthington CLS II, 140 U/mg) and agitated overnight as described by Klagsbrun, (Methods in Enzymology, Vol. VIII). This suspension is then filtered using a 153 gg nylon sieve (Tetko, Elmford, N.Y. 10523). The cells are then removed from the suspension using centrifugation, washed twice with PBS solution and counted with a hemocytometer. The solution is centrifuged at 1800 rpm and the supernatant above the cell suspension removed via suction using a micropipette until the volume of the solution yields a chondrocyte concentration of 5 x 107 cells/cc.

Chondrocytes are expanded in *vitro* in a solution of 10 Ham's F-12 media (Gibco, Grand Island, NY) with 10% fetal calf serum (Gibco), 5 micrograms/ml ascorbic acid, 292 micrograms/ml glutamine, 100 micrograms/ml streptomycin, 40 nanograms/ml vitamin D3 and 100 units/ml penicillin. The cells are incubated at 37°C in the presence of 5% CO_2. Five to eight weeks after initial harvest, the chondrocytes are trypsinized and quantitated using a hemocytometer. The chondrocyte suspension is concentrated to 40 x 10^6 cells/ml in minimal essential media - 199 (Gibco). The cell suspension is mixed with dry alginate powder to form a gel and injected.

Other embodiments and uses of the invention will be apparent to those skilled in the art from consideration of the specification and practice of the invention disclosed herein. All U.S. Patents and other references noted herein for whatever reason are specifically incorporated by reference. The specification and examples should be considered exemplary only with the true scope and spirit of the invention indicated by the following claims.

We Claim:

1. A method for altering a superficial shape of a patient at a modification site comprising the steps of:

a) inserting a hollow tube means into the body of the patient at an insertion site such that an end of said hollow tube is positioned at an injection site beneath the skin of said modification site;

b) positioning a surface shaping means adjacent to the patient's exterior surface at said modification site;

c) deforming the superficial shape at said modification site by forming a seal and applying a vacuum between said surface shaping means and the exterior surface at said modification site; and

d) injecting a biocompatible material subcutaneously at said modification site through said hollow tube.

2. The method of claim 1 wherein the injection site is selected from the group consisting of a subcutaneous site, a subdermal site and an intradermal site.

3. The method of claim 1 wherein said hollow tube is selected from the group consisting of a needle, a cannula, a cannula with a trocar, a catheter, or an angiocatheter.

4. The method of claim 1 wherein said conforming step and said injecting step is performed simultaneously.

5. The method of claim 1 wherein said modification site is a soft tissue defect.

6. The method of claim 5 wherein said soft tissue defect is a skin defect is selected from the group consisting of an acne scar, a developmental defect, a skin depression, a wound scar, a surgical scar, and a surgically caused surface depression.

7. A method of nipple reconstruction comprising the steps of claim 1.

8. The method of claim 1 wherein said biocompatible material is selected from the group consisting of collagen, a fragmented polymer matrix, an alginate matrix, a hydrogel matrix, ceramic beads, crushed gels, and combinations and mixtures thereof.

9. The method of claim 1 wherein said biocompatible material further comprises living cells.

10. The method of claim 9 wherein said cells are selected an autologous cell.

11. The method of claim 9 wherein said cells are selected from the group consisting of fibroblast, myoblast, chondrocyte, endothelial cell, and vascular support cell and mixtures thereof.

12. The method of claim 1 wherein said cell population is a genetically altered to express a desirable characteristic.

13. The method of claim 12 wherein said desirable characteristic is reduced expression of histocompatibility antigens.

14. The method of claim 12 wherein said desirable characteristic is expression of angiogenesis factor.

15. A soft tissue reconstructor, for treating a soft tissue defect in a patient comprising:

surface shaping means comprising a surface of defined shape;

conforming means for deforming a soft tissue comprising said soft tissue defect to approximate the shape of at least a portion of said surface shaping means, said conforming means being attached to said surface shaping means by attachment means;

injection means positioned for injecting a tissue forming composition proximal to said soft tissue defect while said soft tissue is deformed to approximate the shape of at least a portion of said surface shaping means.

16. The soft tissue reconstructor of claim 15 wherein said patient is a human.

17. The soft tissue reconstructor of claim 15 wherein at least a portion of said surface shaping means is a low friction material.

18. The soft tissue reconstructor of claim 15 wherein at least a portion of said surface shaping means is made from a transparent or translucent material.

19. The soft tissue reconstructor of claim 15 wherein said surface shaping means is a rigid surface shaped to conform to a desired exterior contour of the patient.

20. The soft tissue reconstructor of claim 19 wherein said exterior contour is a nipple.

21. The soft tissue reconstructor of claim 19 wherein said exterior contour is selected from the group consisting of a nose, a ear, a lip, and a portion of a face.

22. The soft tissue reconstructor of claim 15 wherein said surface shaping means is adapted to form a vacuum seal with an exterior surface of a patient and wherein said surface shaping means is selected from the group consisting of a flat surface, a curved surface, and a chamber.

23. The soft tissue reconstructor of claim 22 wherein said chamber further comprises a plurality of inserts disposed within said chamber, and wherein each insert adjusts the interior volume of said chamber to conform to a desired exterior contour of a patient.

24. The soft tissue reconstructor of claim 23 wherein one or more of said inserts are porous.

25. The soft tissue reconstructor of claim 15 wherein said conforming means comprises a vacuum source connected to said surface shaping means.

26. The soft tissue reconstructor of claim 25 wherein said vacuum source further comprises a regulator to limit the vacuum within a specific range.

27. The soft tissue reconstructor of claim 15 wherein the injection means is selected from the group consisting of a needle, a cannula, a cannula with a trocar and a catheter.

28. The soft tissue reconstructor of claim 15 wherein the injection means further comprises mechanical positioning means for position said injecting means to insure reproducible placement of the tissue forming composition.

29. A kit for soft tissue reconstruction comprising:

a surface shaping means having a shape that conforms to at least a portion of a desired contour of an exterior surface of a body; and

injection means for depositing a tissue forming composition into tissue.

30. The kit of claim 29 wherein at least a portion of said surface shaping means is transparent or translucent.

31. The kit of claim 29 wherein at least a portion of said surface shaping means is made of a low friction material.

32. The kit of claim 29 wherein said surface shaping means is selected from the group consisting of a flat surface and a chamber.

33. The kit of claim 29 wherein said chamber comprises an interior volume in the shape of a nipple.

34. The kit of claim 29 wherein said surface shaping means is adapted for application of vacuum to an exterior surface of a body.

35. The kit of claim 29 further including attachment means to connect said surface shaping means to a vacuum source.

36. The kit of claim 29 wherein said surface shaping means is coated with sufficient adhesive to allow the attachment of skin in the absence of vacuum.

37. The kit of claim 29 wherein said injection means is selected from the group consisting of a needle, a cannula, or a cannula with a trocar, and a catheter.

38. The kit of claim 29 wherein said injection means further comprises mechanical positioning means for position said injecting means to insure reproducible placement of the tissue forming composition.

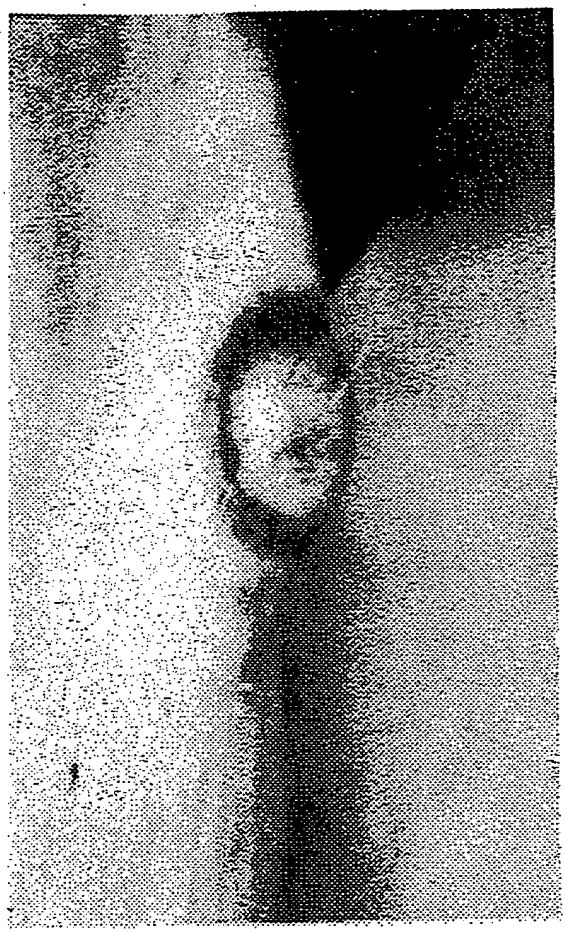


FIG. 1A

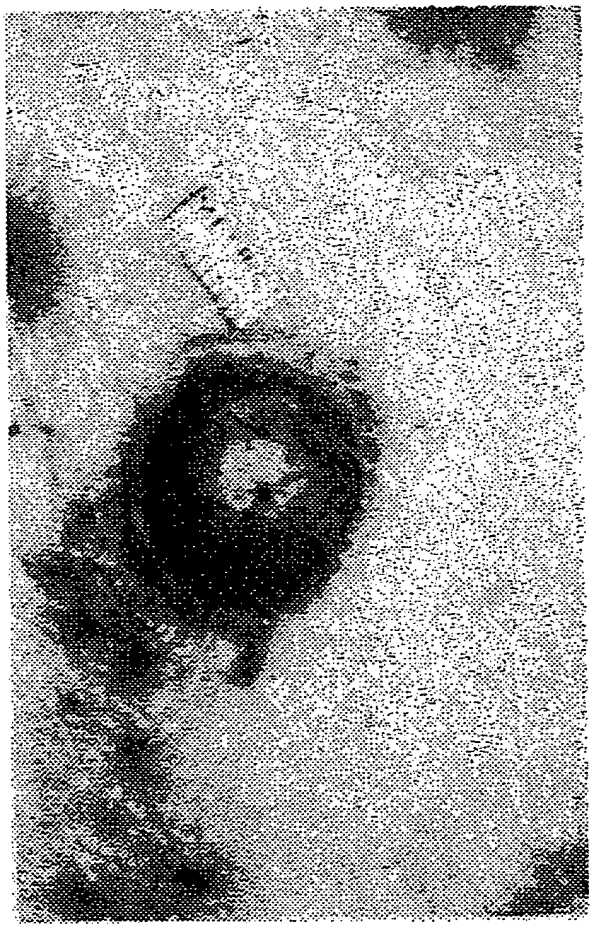


FIG. 1B

FIG. 1C

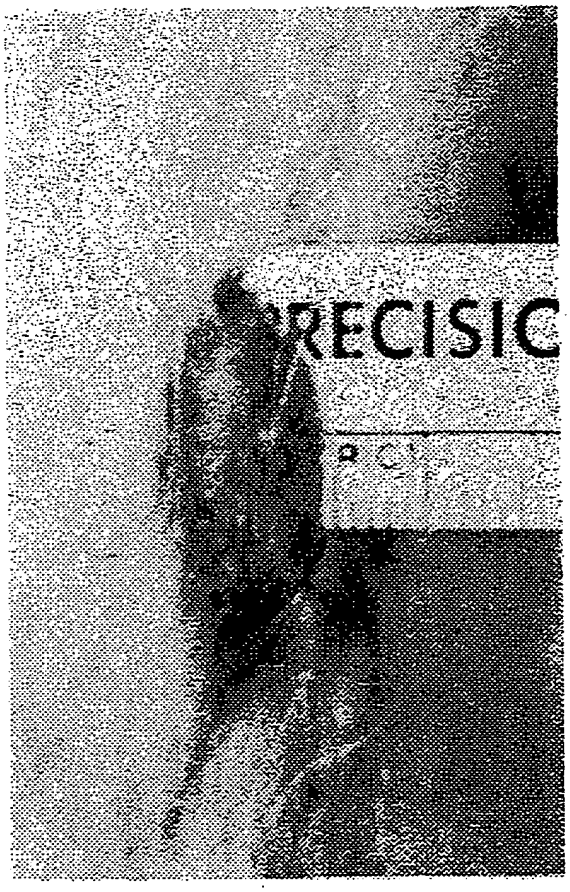


FIG. 1D

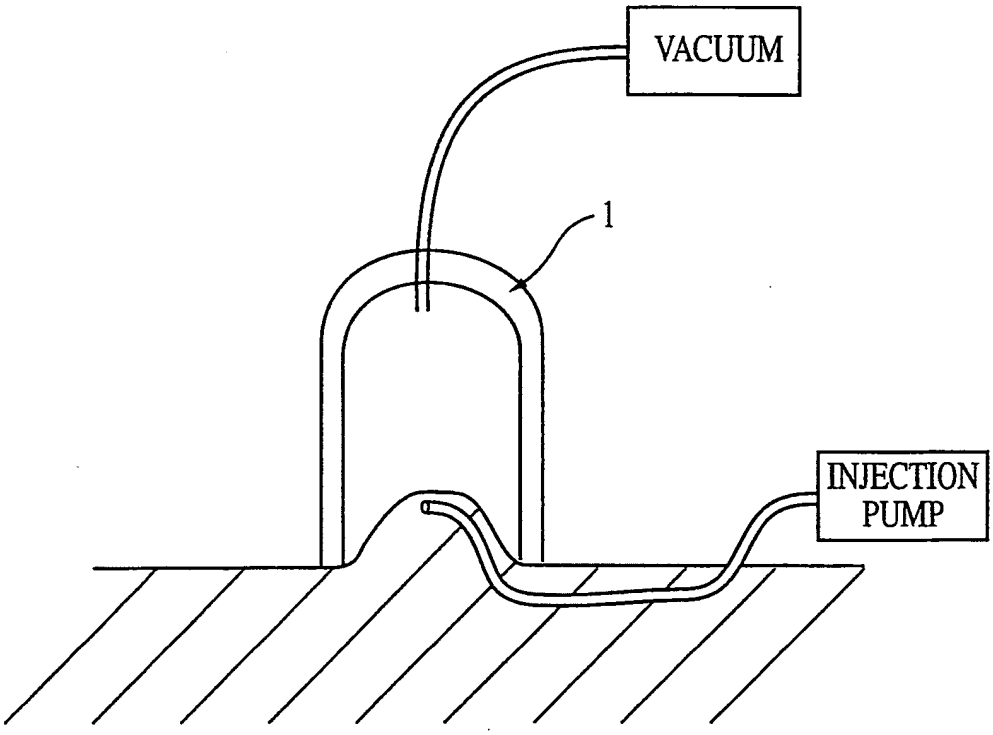


FIG. 2

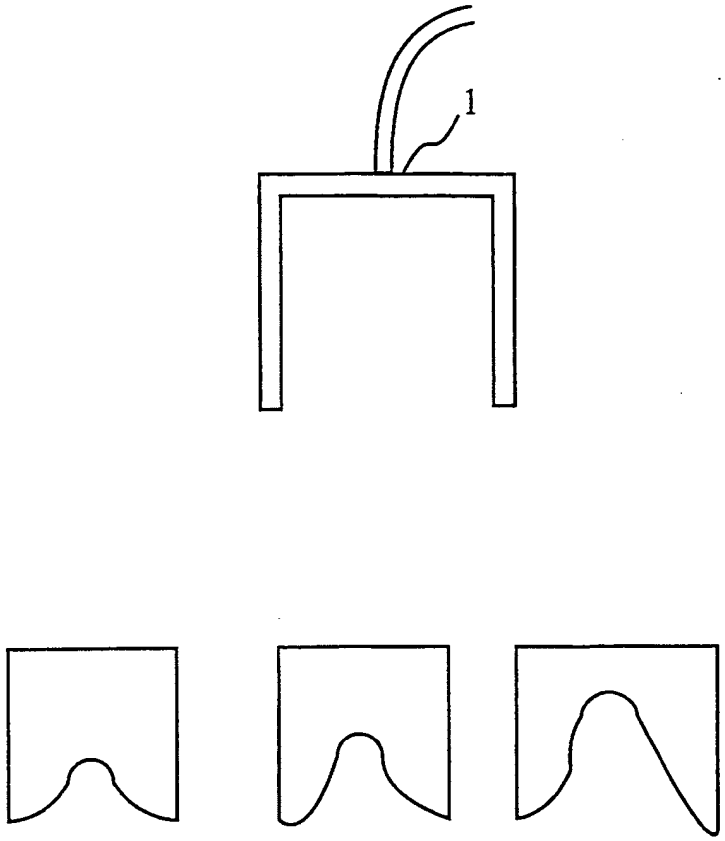


FIG. 3

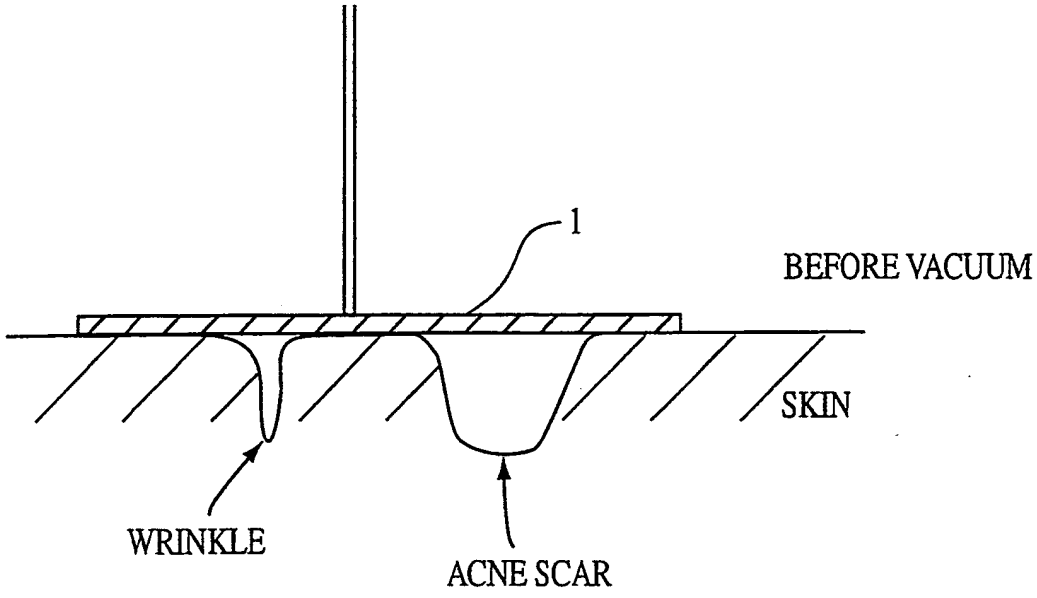


FIG. 4A

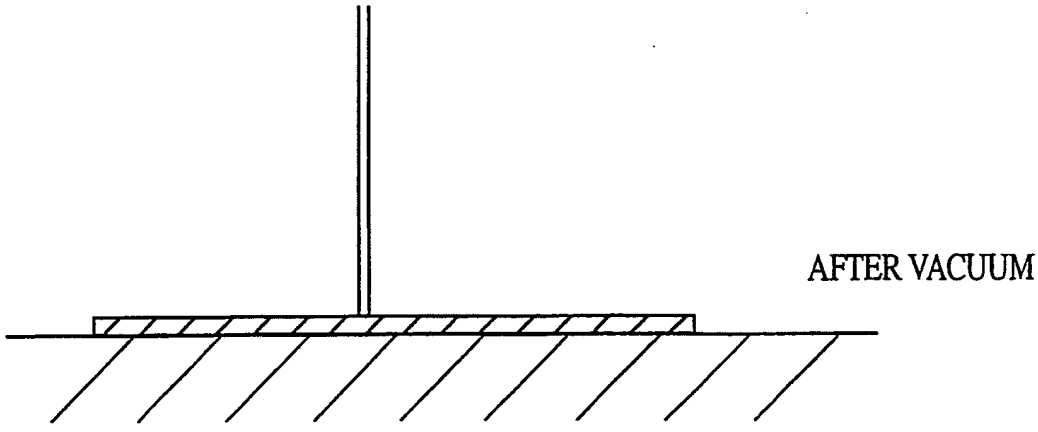


FIG. 4B

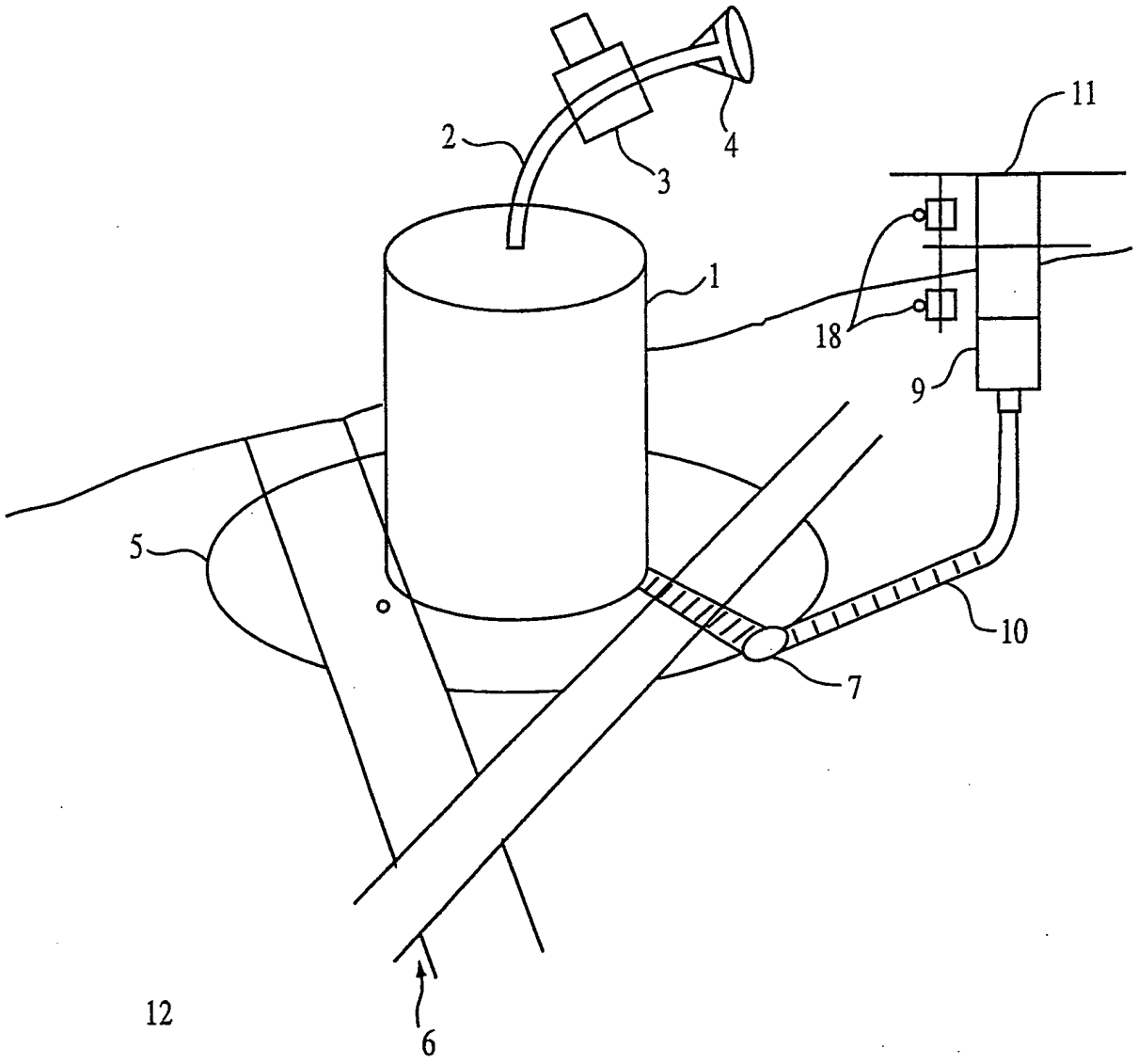


FIG. 5

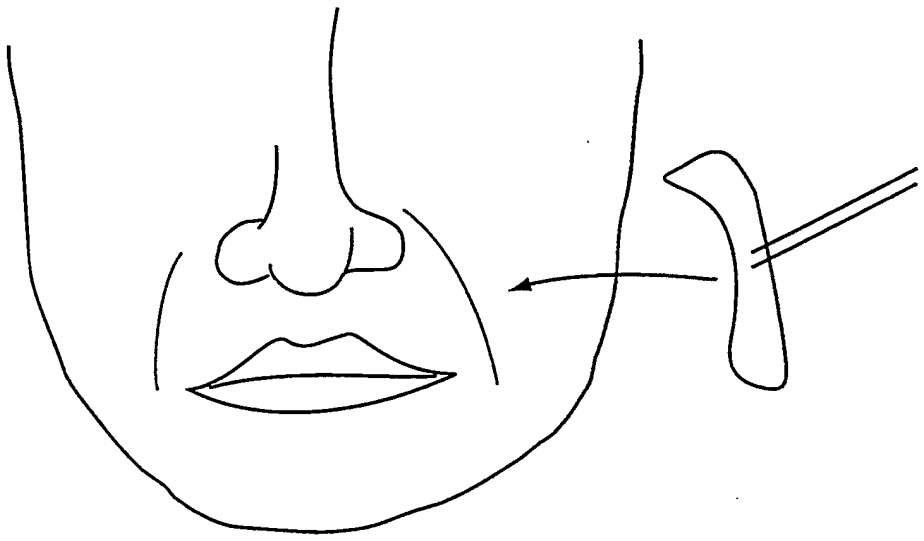


FIG. 6

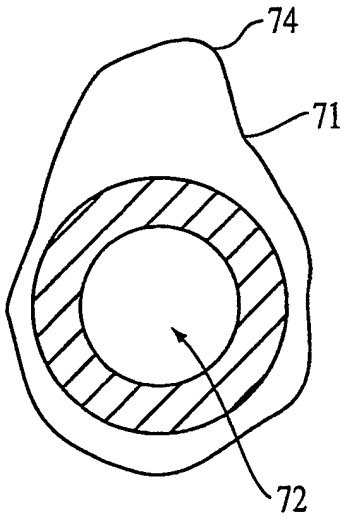


FIG. 7A

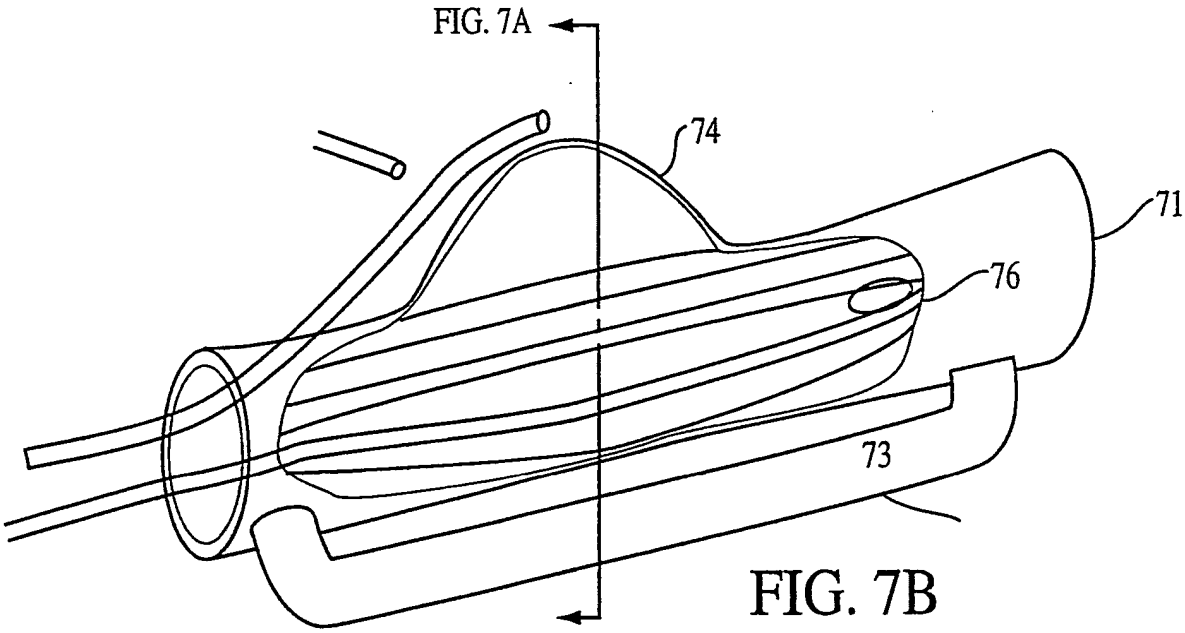


FIG. 7B

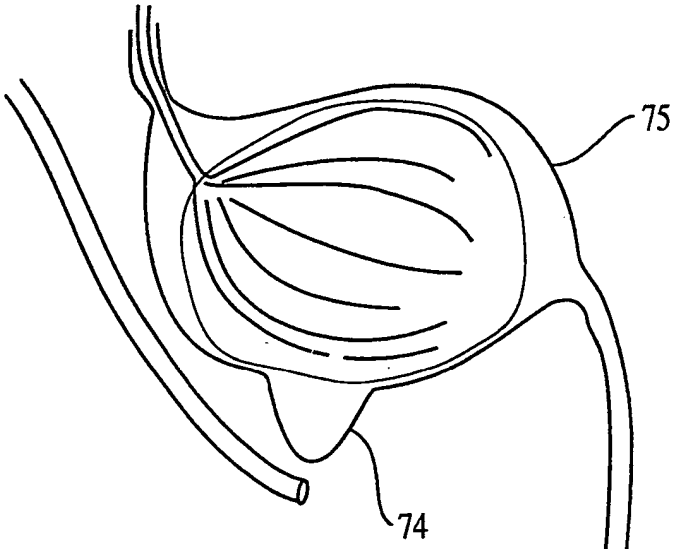


FIG. 7C

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61F2/00 A61K47/00 A61L27/00 A61M5/42 A61B19/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61F A61K A61L A61M A61B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 683 420 A (FOURNET JAMES J ET AL) 4 November 1997 (1997-11-04) column 2, line 32 - line 63; figures	29, 31-35, 37,38
A		15-17, 19,20, 22,25-28
A	US 4 299 219 A (NORRIS JR GEORGE P) 10 November 1981 (1981-11-10) column 4, line 14 - line 19; claims; figures	15,16, 18,19, 22, 25-30, 32,34, 35,37,38

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
6 August 1999	19/08/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Neumann, E

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	WO 89 06944 A (BESINS THIERRY RAINIER ;PHILIPPE BENOIT LOUIS (FR)) 10 August 1989 (1989-08-10) page 9, line 25 - page 11, line 11; figures	15,16, 21, 27-29, 37,38
A	EP 0 692 270 A (COLLAGEN CORP) 17 January 1996 (1996-01-17) column 1, line 41 - line 56; figures column 3, line 35 - column 4, line 23	15,19, 21, 27-29, 37,38
A	BAUER ET AL.: "Möglichkeiten der Vorbehandlung von infizierten Hautweichteildefekten durch Vakuumversiegelung mit PVA-Schaumstoff" HANDCHIR. MIKROCHIR. PLAST. CHIR., 30 January 1998 (1998-01-30), pages 20-23, XP002111515 Hamburg, DE abstract	

INTERNATIONAL SEARCH REPORT

ernational application No.
PCT/US 99/06745

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 1-14
because they relate to subject matter not required to be searched by this Authority, namely:
Rule 39.1(iv) PCT-Method for treatment of the human or animal body by surgery

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5683420 A	04-11-1997	US 5800534 A	01-09-1998
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